# THE INFLUENCE OF LIGHT ON THE SIZE OF AGGREGATIONS IN DICTYOSTELIUM DISCOIDEUM

THEO M. KONIJN[1] AND KENNETH B. RAPER

*Departments of Bacteriology and Botany, University of Wisconsin, Madison, Wisconsin 53706*

Fruiting structures of the Dictyosteliaceae formed in light are smaller and more numerous than those produced in comparable cultures incubated in darkness (Potts, 1902; Harper, 1932; Raper, 1940; Heller and Miles, 1961; Shaffer, 1961; Kahn, 1964). Since the size of a fructification, or sorocarp, is dependent to a large extent upon the number of cells that enter an aggregation, it is logical to seek the bases for this behavior in the aggregative process *per se*. Bonner and co-workers (Bonner and Dodd, 1962; Bonner and Hoffman, 1963) have reported that for certain species of *Dictyostelium* the size of the aggregation territory remains the same in cultures grown under constant environmental conditions. They believe it possible, as does Shaffer for *Polysphondylium violaceum* (Shaffer, 1961), that an inhibitory substance diffuses outward from the first-formed centers and prevents the formation of additional ones, thus determining the disposition of developing aggregations. To this putative factor they have applied the term "spacing substance." More recently, Kahn (1964) has suggested that cell aggregation in *Polysphondylium pallidum* may be inhibited in darkness by a center-suppressing factor, the effect of which is erased by illumination. For a summary of published information and opinion prior to 1962 regarding the process of cell aggregation in the Dictyosteliaceae, the reader is referred to Shaffer's comprehensive review (1962), "The Acrasina."

The effect of different light conditions on the time of aggregation in *Dictyostelium discoideum* has been investigated (Konijn and Raper, 1965). But no studies have been made to determine at what time during the preaggregative stage the existing light conditions influence the number of aggregations formed (and by inference the size of aggregation territories) or the number of fruiting bodies that subsequently develop. The present study attempts to assess the effect of light during the preaggregative stage on the number of aggregations that subsequently arise, and to correlate differences in the size of such aggregations in light and in darkness with possible changes in the activity of, or the cellular responses to, the chemotactic substance(s) secreted by the converging myxamoebae.

## Materials and Methods

*Dictyostelium discoideum* Raper, NC-4(H), a haploid strain derived from the diploid stock, NC-4, was the culture most used during this research. The myxamoebae were grown in either light or darkness and on either a solid medium

[1] Present address: Hubrecht Laboratory, Universiteitscentrum de Uithof, Utrecht, the Netherlands.

(Bonner, 1947) in association with *Escherichia coli* #281, *E. coli* B/r or *Aerobacter aerogenes* #900, or in shaken tube cultures with pregrown *E. coli* B/r according to the technique of Gerisch (1959). The details of harvesting the myxamoebae were given in our previous report (Konijn and Raper, 1965).

Populations of two sizes and densities were employed in the present study:

(1) For investigating the number and size of aggregations that would develop under different light conditions, washed myxamoebae were resuspended in distilled water, or in Bonner's salt solution (Bonner, 1947), at a dilution of $4 \times 10^5$ cells/ml. and deposited on non-nutrient agar (1.5%) as 0.1-ml. aliquants. Three such drops were implanted per Petri dish and, after having spread on the agar surface, covered areas 1.5 to 1.9 cm. in diameter. The resultant cell density was usually about 200 to 250 myxamoebae per/mm.$^2$, there being some increase in the cell populations after deposition (see Konijn and Raper, 1961).

(2) For investigating the influence of light on cell attraction, *minute* droplets of a much denser suspension ($1.5 \times 10^7$ cells/ml.) were deposited on a soft gel made with highly purified agar and Bonner's salt solution (Konijn and Raper, 1961). The number of cells per population was 800 to 1600, and the areas covered by the droplets were approximately 0.5 mm. in diameter. Depending upon the time of deposition, populations were designated as either "attracting" or "responding." From 150 to 200 droplets containing attracting populations were first deposited on the surface of an agar plate with hand-drawn micropipettes, while an equal number of similar droplets containing responding populations were deposited later at distances of 400 to 1200 $\mu$ from the former. The concentration of the agar used to form the gel was *ca.* 0.5%, or sufficient to yield a rigidity of 35 to 40 grams expressed as the weight required to cause the end of a microscope slide to break the agar surface (Konijn and Raper, 1961). For a particular experiment, a single cell suspension was used as the source of both attracting and responding populations, the suspension being held in a refrigerator until the latter were placed on agar.

The light source employed was "cool white" fluorescent tubes, and the light intensity was *ca.* 60 foot candles at the level of the agar surface on which the myxamoebae were deposited. The incubation temperature was $23 \pm 1°$ C.

## Results

### 1. *The influence of light on the number and size of developing aggregations and sorocarps*

Myxamoebae of *Dictyostelium discoideum,* NC-4(H), were grown on agar in darkness and harvested in the preaggregative stage. After removal of excess bacteria by centrifugation, the cells were resuspended, deposited on non-nutrient agar and incubated under different light conditions. The total number of fruiting organizations formed within the populations was recorded after 25 hours. As shown in Figure 1, more and smaller aggregations and fruiting structures were formed in constant light than in constant darkness. Since only a small minority of the myxamoebae stayed outside the developing aggregations in either case, such cells could not account for the lower number produced in the dark. Of special interest was the influence of an initial period of dark incubation of varying duration. The number of sorocarps formed in light gradually decreased as an initial dark period

was increased, and the lowest number developed if a dark period optimal for early aggregation (*e.g.*, 8 to 9 hours darkness) preceded exposure to light. Myxamoebae that were transferred from darkness to light shortly before aggregation began, or in the early aggregative stage, usually produced a near maximum to maximum number of fruiting structures, as for example in plates incubated in darkness for 12 or 13 hours. The number of fruiting structures formed under similar light conditions varied considerably in different experiments. After an initial dark period of 14 hours, aggregations in some experiments were already well advanced, and the

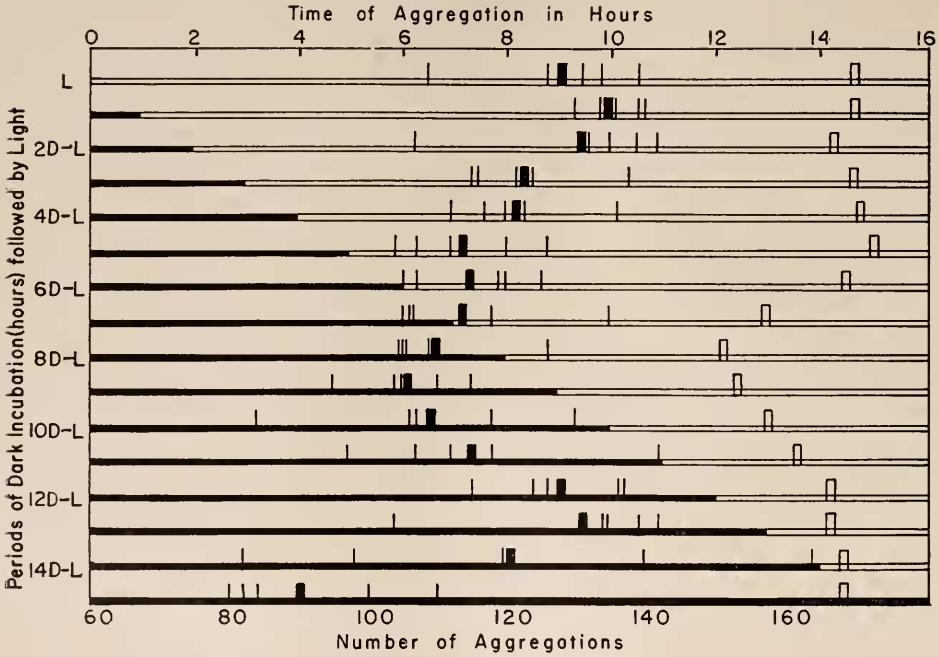



FIGURE 1. The effect of an increasing initial dark period on the number of aggregations and sorocarps in *Dictyostelium discoideum*, NC-4(H). The myxamoebae were grown in the dark on a solid medium. The results of five experiments are included. ═ Light. ■ Darkness. | Number of aggregations per population in individual experiments. Each mark represents the average of 6 drops. ▮ Average number of aggregations per population in all experiments. ┌┐ Average time of aggregation.

final count of fructifications in such plates was close to the total number formed in complete darkness. In other experiments, the myxamoebae were in a less advanced stage and in these the number of aggregations was increased substantially by exposure to light. Variation in the diameters of the drop areas due to minor differences in the non-nutrient agar may have contributed somewhat to the inconstant behavior. However, such variation should not have been greater in plates incubated for 14 hours in darkness than in the other series.

The same general approach was used in a second set of experiments except that the myxamoebae were grown in shaken cultures in the dark. In these tests, variation caused by differences in the areas covered by the drops, or by a possible "edge

effect" at the boundaries of the drops, was minimized by counting only the number of fruiting structures in an area 1.0 cm. square at the center of each population. Counts obtained in this way were roughly proportional to those obtained in the earlier experiments when all the sorocarps that developed within a drop were counted (Fig. 2). The onset of aggregation occurred somewhat earlier than with

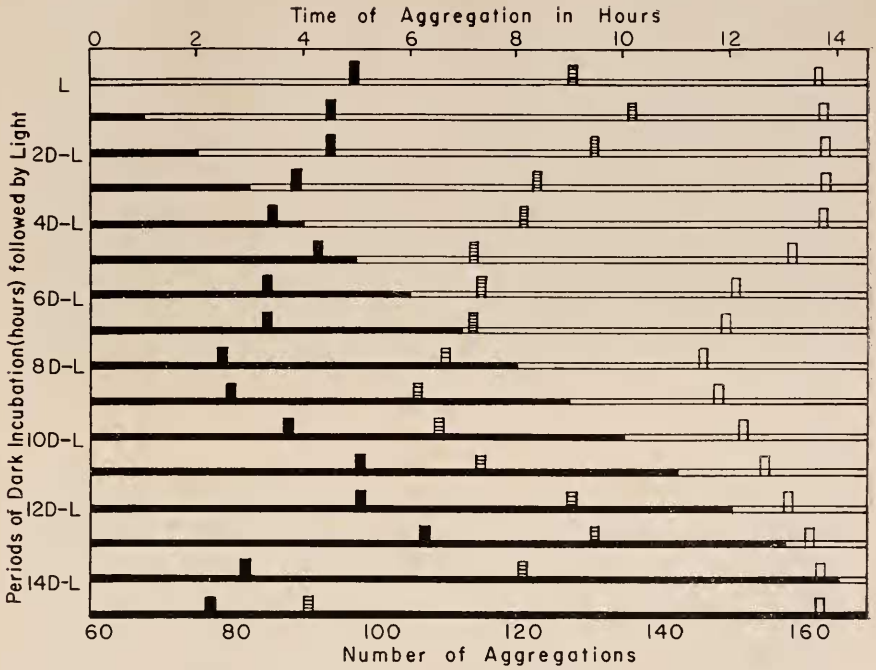



FIGURE 2. A comparison of the numbers of aggregations in entire populations of *Dictyostelium discoideum*, NC-4(H), and from areas 1 cm. square at centers of populations of comparable size and density. The myxamoebae for the latter tests were grown in the dark in a liquid medium. The results of four experiments are averaged. ═══ Light. ▬▬ Darkness. ■ Average number of aggregations in centrally placed areas 1 cm. square. Each mark represents the average of 24 determinations. ┌┐ Average time of aggregation in the four experiments. ≣ Average numbers of aggregations in entire populations of comparable size and density. (Data from Fig. 1.)

myxamoebae dark-grown on the solid medium, this difference being as much as two hours in cultures incubated in the dark for 5 or 6 hours prior to being transferred to light.

Other species and strains were examined in a third set of experiments. In *Dictyostelium purpureum*, WS 321, and in *Polysphondylium pallidum*, WS 320, the number of aggregations was much reduced when the populations of myxamoebae were transferred from light to darkness at an early aggregative stage. If the cells were first kept in the dark for a few hours and later exposed to light, the number of aggregations increased if the transfer took place at or near the beginning of aggregation. Also, in *D. discoideum*, strain Acr 12, aggregations

occurred earlier and in greater numbers in light than in darkness. Thus inter- and intraspecific differences in response to light must occur since cell populations of low density (200–250 myxamoebae/mm.$^2$) were employed, as for *D. discoideum*, NC-4(H).

2. *The influence of light during the preaggregative stage on the rate of increase in the number of aggregations*

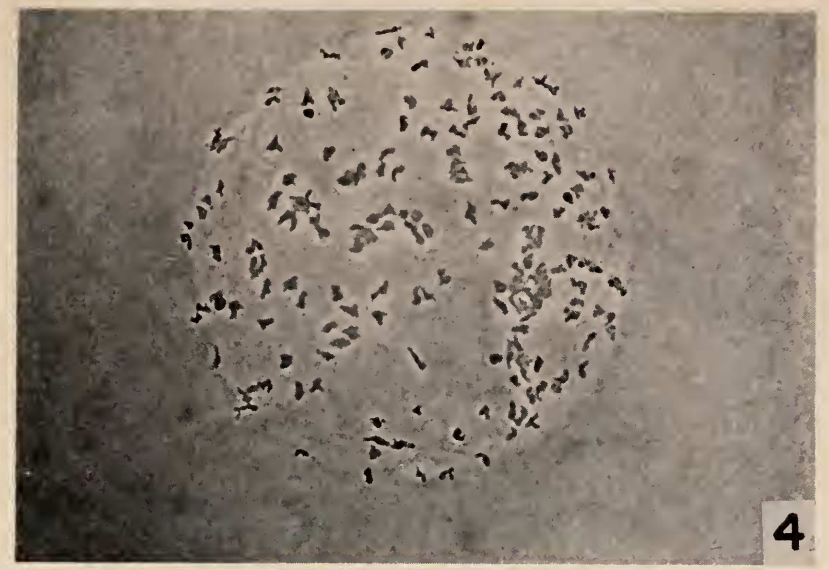
Myxamoebae of *Dictyostelium discoideum*, NC-4(H), were grown in shaken cultures in the dark, harvested, washed and deposited as 0.1-ml. drops on non-nutrient agar. Surveys at half-hour or hourly intervals after the cells started to aggregate indicated that the number of aggregations increased slowly if an initial


FIGURE 3. The effect of an increasing initial dark period on the rate of increase in number of aggregations. L: Light; D: Darkness; D–L: the drops were in darkness for the number of hours indicated before exposure to the light.

dark period was lacking, or if this was insufficient to induce early aggregation (Fig. 3). After a dark period of 8 hours or longer, however, the number of aggregations increased rapidly, and within a period of one to three hours the final number was reached. Primary aggregations in populations exposed to light after a dark incubation of 10–13 hours were relatively large and the increase in number of sorocarps formed in such plates resulted, in substantial part, from a breaking up of the streams of pre-existing aggregations into smaller pseudoplasmodia. The sample curves presented in Figure 3 are taken from a much more extensive series twice confirmed in which periods of light and dark incubation were varied at increments of less than four hours.

3. *The influence of light on chemotaxis*

Information relating to the phenomenon of chemotaxis was obtained by observing the behavior of small populations of myxamoebae when deposited on the suface



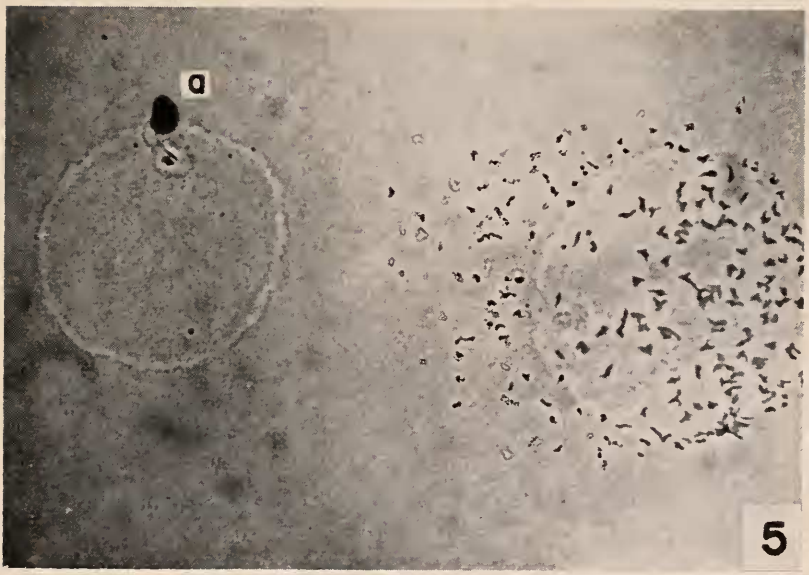




FIGURE 4. A small population of myxamoebae deposited on washed non-nutrient agar of low rigidity. Without attracting forces from the outside the myxamoebae stay inside the boundary of the drop. × 140.

FIGURE 5. Myxamoebae in a responding population attracted outside the boundary of the original drop by an aggregation (a) in the neighboring drop. The myxamoebae move toward the attracting aggregation by wriggling through the agar. The rigidity of the agar surface was the same as in Figure 4. × 100.

of washed, non-nutrient agar of low rigidity (Konijn and Raper, 1961). The soft agar gel provided a means of measuring interpopulational responses since it was rigid enough to keep all the cells within the confines of the drop in the absence of an extraneous stimulus (Fig. 4), but was, at the same time, sufficiently soft to allow the myxamoebae to move outside the drop boundary if attracted by a chemotactic stimulus secreted by a neighboring population. Fortunately for our purposes, the myxamoebae that were attracted beyond the edge of such a drop moved *into* the agar (Fig. 5) and did not return to their "home" drop until its residual cells formed their own aggregates. Thus data relating to the effect of chemotactic stimuli could be obtained by employing droplets of cells of one preaggregative "age" to act as attractors and cells that were less mature in point of time to serve as responders. Drops containing attracting and responding myxamoebae were

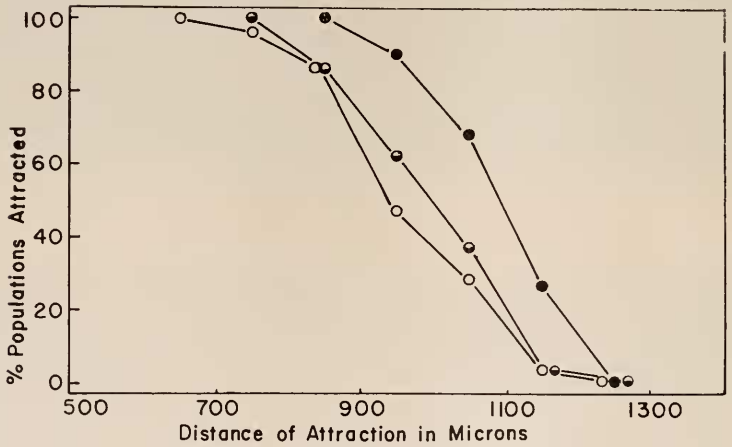



FIGURE 6. The percentages of myxamoeba populations that showed a response to developing aggregations under different light conditions, and the distances between the nearest margins of these populations and the aggregations that attracted them. Graphic representation of data contained in Table I. ○: Constant light. ◒: Constant light, but with plates sealed after the responding drops were deposited on the agar. ●: Constant darkness.

deposited on the agar surface at different distances from each other and the plates were then incubated in either light or darkness. The distance over which attraction could take place was taken as a measure of the strength of the stimulus produced by the population of aggregating myxamoebae. This distance was measured not between the proximal edges of the two drops but from the *center* of the developing aggregation in the attracting drop to the nearest margin of the responding drop. The response was considered as positive when two or more cells moved outside the edge of the responding drop toward the attracting population. Responding myxamoebae rarely moved more than 500 $\mu$ outside their "home" drops.

For the actual tests, minute droplets of a dense suspension of cells pregrown on a solid medium were deposited on low-rigidity washed agar and occupied areas approximately 0.5 mm. in diameter. In preliminary experiments, responding populations plated three hours after the attracting populations showed less response

when incubated in the light than in darkness. Fifty per cent of the attracting small populations incubated in the light aggregated after about 8–10 hours, which was on average one hour earlier than those in the dark, and it was thought that responding cells that were themselves nearer in time to aggregation might have an increased sensitivity to chemotactic substances. In order that the "physiological age" of the responding drops in light and in dark might be more nearly identical at the time aggregation began in their counterpart attracting drops, the responding cells to be incubated in the dark were deposited one hour later than those to be incubated in the light. The influence of light on attraction by aggregating myxamoebae is graphically presented in Figure 6. Attraction in the dark was observed to occur, on the average, over a longer distance than in the light (Table I). For example, 50% of the illuminated populations showed a response over a distance of 940 $\mu$, while an equal percentage of those incubated in the dark responded over a distance of 1090 $\mu$.

TABLE I

*Attraction of myxamoebae in populations of responding cells by developing aggregations in plates incubated in light, in darkness, and in light with plates sealed. The 2056 populations represented in this table were tested in three different experiments*

| Distance between aggregations and the responding populations | Light | | | Light, sealed | | | Darkness | | |
|---|---|---|---|---|---|---|---|---|---|
| | + | − | % | + | − | % | + | − | % |
| 600– 700 $\mu$ | 17 | 0 | 100 | | | | | | |
| 700– 800 $\mu$ | 47 | 2 | 96 | 30 | 0 | 100 | 17 | 0 | 100 |
| 800– 900 $\mu$ | 126 | 22 | 85 | 137 | 23 | 86 | 153 | 0 | 100 |
| 900–1000 $\mu$ | 98 | 111 | 47 | 125 | 77 | 62 | 233 | 25 | 90 |
| 1000–1100 $\mu$ | 45 | 118 | 28 | 49 | 84 | 37 | 159 | 76 | 68 |
| 1100–1200 $\mu$ | 2 | 61 | 3 | 2 | 57 | 3 | 20 | 58 | 26 |
| 1200–1300 $\mu$ | 0 | 27 | 0 | 0 | 22 | 0 | 0 | 33 | 0 |

Code: + Test populations responding to an aggregation in the "attracting drop"; − Test populations not responding to an aggregation in the "attracting drop"; % Percentage of test populations responding.

A possible decrease of humidity in plates incubated in the light could have affected the agar surface and consequently limited the movement of the responding cells outside the edge of the drop. For this reason, control plates in the light were sealed with masking tape to prevent evaporation and to reduce any possible gaseous exchange which might influence aggregation, as has been reported by Bonner and Hoffman (1963). The distance over which attraction occurred in sealed plates in three different experiments was equal to or slightly greater than that observed in the unsealed, light-incubated plates, but it was never equivalent to that recorded in the dark (Fig. 6).

## DISCUSSION

When an initial dark period optimal for early aggregation in *Dictyostelium discoideum*, NC-4(H), was employed (see also Konijn and Raper, 1965), the number of sorocarps was less than in constant light and greater than in constant

darkness. A further increase in the period of dark incubation resulted in a delay of aggregation and an increase in the number of sorocarps, particularly, if the transfer to light occurred near or at the onset of aggregation. The number of sorocarps, however, was not necessarily related to the time of aggregation. Myxamoebae of *D. discoideum* incubated in continuous light aggregated slightly later than cells kept in darkness, but formed sorocarps more abundantly. Light could exert its effect by a stimulation of center formation, or by an inhibition of the spacing substance (Bonner and Hoffman, 1963), *e.g.,* by reducing the sensitivity of cells to it. It is questionable whether a gaseous spacing substance determines the number of aggregations and consequently influences the number of fruiting structures in this species, for Bonner and Hoffman (1963) noticed that the gaseous spacing substance that has such a pronounced effect on *D. mucoroides* does not affect the myxamoebae of *D. discoideum,* although the latter are able to produce a gaseous substance that influences the spacing in other species.

The increase in the number of sorocarps produced when myxamoebae are exposed to light at an early aggregative stage is dependent, at least in part, on a delicate balance within the aggregations. For example, if populations in the process of aggregating were placed under fluorescent light, the aggregates already formed would sometimes break up into several smaller pseudoplasmodia. At other times the binding forces within the aggregations were strong enough to prevent severance of streams when exposed to light, and no significant increase in the number of sorocarps occurred.

When a very few aggregations are formed early, as after a short initial dark period (Konijn and Raper, 1965), one would expect these centers to extend their spheres of attraction over large areas and subsequently to produce only a few and large sorocarps. However, this does not occur; instead many aggregations are formed although the increase in number occurs slowly. When all aggregations appear at about the same time, as after a long initial dark period, their size is generally larger and their number less.

The limited size of aggregations in the former populations may be due to a reduced acrasin secretion per cell as the size of the aggregate increases, and, after a certain acrasin concentration has been reached, additional cells entering the aggregate may not further increase the level of acrasin. This concentration may be sufficient to attract all cells within the aggregate's territory. This would conform with Shaffer's observation that acrasin sources of various sizes seem to secrete at the same concentration (Shaffer, 1957), and would support his assumption that the acrasin secretion per cell is inversely related to the size of the aggregate (Shaffer, 1962).

It is a common observation that streams of myxamoebae flowing into centers in darkness are longer than those in light. This may result in part from an increased stickiness of the cells in darkness that is reflected in somewhat longer streams, which in turn, since these also secrete acrasin, attract additional sensitive myxamoebae to further enlarge the aggregations. But this is only one of many ways in which light may act on aggregating myxamoebae.

Light may depress the formation of acrasin, or its precursors, or have a regulating action on the secretion of acrasin, *e.g.,* by changing the permeability of the cell membranes. If light affects the attraction of the cells it may do so by an

inactivation of acrasin, or by altering the sensitivity of the myxamoebae to it. That inactivation of acrasin, presumably enzymatic, does occur has been shown by Shaffer (1956). Another effect of increased inactivation of acrasin could be the occurrence of a steeper gradient, which would favor earlier aggregation. If inactivation of acrasin is enhanced by light, attraction should occur over a shorter distance in light, hence results in the formation of smaller aggregations and sorocarps. Smaller sorocarps were actually observed in plates incubated in the light, and a reduced attraction of responder cells in populations exposed to light was observed in an assay system in which attracting and responding cells were separated from each other by different distances at the time attraction occurred.

This work was made possible by research grants from the National Institutes of Health (CA 02119-09), U. S. Public Health Service, and the National Science Foundation (G-24953).

## Summary

1. Myxamoebae of *Dictyostelium discoideum*, NC-4(H), were pregrown in the dark on *Escherichia coli* or *Aerobacter aerogenes*, washed and deposited on non-nutrient agar. Populations incubated in constant light produced more and smaller aggregations and sorocarps than similar populations incubated in the dark. If populations were incubated in darkness for several hours and then transferred to light, the number of aggregations was reduced and the dimensions of these and the resulting sorocarps were correspondingly greater. The rate of increase in the number of aggregations was most rapid if the myxamoebae were exposed to a long initial dark period followed by light.

2. The chemotactic response of myxamoebae incubated in light or darkness was studied by depositing "attracting" and "responding" cells in separate small populations at predetermined distances from each other. The sphere of attraction by myxamoebae aggregating in light was found to extend over a shorter distance than that of cells aggregating in darkness. Among other possibilities, inactivation of the attracting substance(s) in the light may account for reduced attraction, hence result in smaller aggregations.

## LITERATURE CITED

Bonner, J. T., 1947. Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. *J. Exp. Zool.*, **106**: 1–26.

Bonner, J. T., and M. R. Dodd, 1962. Aggregation territories in the cellular slime molds. *Biol. Bull.*, **122**: 13–24.

Bonner, J. T., and M. E. Hoffman, 1963. Evidence for a substance responsible for the spacing pattern of aggregation and fruiting in the cellular slime molds. *J. Embryol. Exp. Morph.*, **2**: 571–579.

Gerisch, G., 1959. Ein Submerskulturverfahren für entwicklungsphysiologische Untersuchungen an *Dictyostelium discoideum*. *Naturwiss.*, **23**: 654–656.

Harper, R. A., 1932. Organization and light relations in *Polysphondylium*. *Bull. Torrey Bot. Club*, **59**: 49–84.

Heller, S. A., and M. G. Miles, 1961. The effect of humidity and light on the concentration and distribution of sorocarps of *Dictyostelium purpureum*, str. 2. Senior thesis, Princeton.

KAHN, A. J., 1964. The influence of light on cell aggregation in *Polysphondylium pallidum*. *Biol. Bull.*, **127**: 85–96.

KONIJN, T. M., AND K. B. RAPER, 1961. Cell aggregation in *Dictyostelium discoideum*. *Develop. Biol.*, **3**: 725–756.

KONIJN, T. M., AND K. B. RAPER, 1965. The influence of light on the time of cell aggregation in the Dictyosteliaceae. *Biol. Bull.*, **128**: 392–400.

POTTS, G., 1902. Zur Physiologie des *Dictyostelium mucoroides*. *Flora*, **91**: 281–347.

RAPER, K. B., 1940. Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J. Elisha Mitchell Sci. Soc.*, **56**: 241–282.

SHAFFER, B. M., 1956. Acrasin, the chemotactic agent in the cellular slime moulds. *J. Exp. Biol.*, **33**: 645–657.

SHAFFER, B. M., 1957. Properties of slime-mould amoebae of significance for aggregation. *Quart. J. Micr. Sci.*, **98**: 377–392.

SHAFFER, B. M., 1961. The cell founding aggregation centres in the slime mould *Polysphondylium violaceum*. *J. Exp. Biol.*, **38**: 833–849.

SHAFFER, B. M., 1962. The Acrasina. Adv. in Morphogenesis. Vol. II. pp. 109–182. Academic Press Inc. New York and London.